Considered
5/29/07

PATENT

AMD
5/29/07

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Joseph M. Penninger
Michael A. Crackower

Serial No.: 10/518,599

Filed: May 31, 2005

For: ACE2 ACTIVATION FOR TREATMENT OF HEART, LUNG AND KIDNEY DISEASE AND HYPERTENSION

Group Art Unit: 1632

Examiner: Anoop Kumar Singh

Atty. Dkt. No.: SONN:064US

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

March 20, 2007
Date

Travis M. Wohlers

DECLARATION OF DR. NIKOLAUS NEU UNDER 37 C.F.R. § 1.132

I, Nikolaus Neu, hereby declare as follows:

1. I am an Austrian citizen residing in Innsbruck, Austria. I am the head of the pediatric intensive care unit at University Hospital Innsbruck. I have extensive research experience in the fields of cardiology and immunology. I have published scientific papers on topics such as acute pulmonary arterial hypertension in acute lung injury, peptide-induced inflammatory heart disease, and heart disease linked through antigenic mimicry. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. I have reviewed the specification of the above-reference application, the amended set of claims, and the Office Action dated October 20, 2006 ("the Action"). I understand that the Action rejected claims 67-69, 73, and 98-103 for lack of enablement and for failure to comply with the written description requirement. I do not find this to be the case based on my review of the specification.

3. The present specification discloses that ACE2 is a critical negative regulator of the renin-angiotensin system (RAS) (paragraph bridging pages 2-3). ACE2 cleaves angiotensin I (Ang I) to generate Ang 1-9, and it cleaves angiotensin II (Ang II) to generate Ang 1-7 (Specification, p. 29, ln. 10-16). The effects of Ang II are summarized in the attached review articles by Danilczyk *et al.* (2004 and 2006) (attached as Exhibits 2 and 3, respectively). Therein it is reviewed that Ang II is a vasoconstrictor, promotes cardiomyocyte hypertrophy, fibroblast proliferation, cardiac and cardiomyocyte contractility and regulates glomerular hemodynamics - whereas Ang 1-7 is a vasodilator, inhibits cell growth, regulates sodium and water flux and reduces glomerular filtration (e.g. Danilczyk *et al.*, 2006, table p. 465). As mentioned above, Ang II is converted to Ang 1-7 by ACE2 thereby reducing the effects of Ang II and increasing the effects of Ang 1-7. Loss of ACE2 results in an increase in Ang II, which was shown in the mouse knock-out model in the present specification (Specification, p. 38, ln. 13-26).

4. The present specification discloses that various cardiac, lung, and kidney diseases are associated with an ACE2 decreased state (*see e.g.*, p. 2, ln. 28 to p. 3, ln. 6). This disclosure is supported by rat and mouse model studies. For example, decreased ACE2 mRNA and protein levels were observed in the kidneys of a hypertensive rat model (Specification, p. 32, ln. 21 – p. 33, ln. 22). The specification also describes an ACE2 knockout mouse, which is used to model

the ACE2 decreased state. In studies on the ACE2 knockout mouse, it was observed that loss of ACE2 leads to detrimental effects in the kidneys (p. 36, ln. 8-11), heart defects (p. 36, ln. 14 – p. 38, ln. 12), and increases the susceptibility of the lungs to injury (p. 40, ln. 12-20). The specification teaches that an ACE2 decreased state, such as cardiac, lung, and kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (p. 9, ln. 10-15). The specification further teaches that this agent may be an Ace2 protein or fragments thereof (p. 9, ln. 16-25).

5. The specification also discloses the evolutionary conservation of ACE2 structure and activity among mammals, as well as other organisms. FIG. 1A shows an alignment of the amino acid sequences of ACE2 from human, rat, and mouse, which illustrates the identities and similarities between these sequences. Previous results in *Drosophila* showed that a P-element mutation associated with the ACE homologue, ACER, results in a severe and lethal defect of heart morphogenesis, which is further evidence that ACE/ACE2 functions in the heart have been conserved through evolution (Specification, p. 30, ln. 28 to p. 31, ln. 2). I have also reviewed a publication entitled "Structure, Evolutionary Conservation, and Function of Angiotensin- and Endothelin-Converting Enzymes" (Macours *et al.*, *International Review of Cytology*, 239:47-97 (2004); IDS reference C63), and the results of a BLAST search (IDS reference C60) of the ACE2 substrate, Ang II, which shows that Ang II is present in numerous mammals including *Pan troglodytes, Mus musculus, Homo sapiens, Callithrix jacchus, Gorilla gorilla, Canis familiearis, Macaca mulatta, Rattus norvegicus, Ovis ammon,* and *Pongo pygmaeus*. The Macours *et al.* publication and the BLAST search results provide further evidence of the evolutionary conservation of ACE2 and its substrate Ang II. In view of this evidence, I find that ACE2

structure and function is conserved among mammals, and I would expect that the currently claimed method could be practiced in any mammal.

6. The use of protein therapy in the treatment of diseases is well-known in the medical field. The publication *Scientific Considerations Related to Developing Follow-On Protein Products*, 2004, which is cited in the present Office Action, illustrates this point. For example, this publication mentions the drugs Epogen®, which is a protein therapy based on human erythropoietin; and Neupogen®, which is a protein therapy based on granulocyte colony-stimulating factor (*see,* p. 1, 2nd para.). As a further example, the publication notes that six companies manufacture FDA-approved versions of human growth hormone (paragraph bridging pages 5-6). Since ACE2 is an endogenous protein in mammals and the present specification discloses the physiological role of ACE2, it would require only routine clinical studies to administer a therapeutically effective amount of an ACE2 polypeptide to an mammal having hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis, renal failure, and/or lung disease, in order to treat the mammal as recited in the current claims.

7. I have also reviewed the reference by Imai *et al.* (*Nature*, 436:112-116 (2005); IDS reference C61), which further demonstrates that scientists can practice the currently claimed method based on the information provided in the present specification. Imai *et al.* showed that injecting ACE2 knockout mice or acid-treated wild-type mice with a recombinant human ACE2 protein protected the mice from severe acute lung injury (p. 112, col. 2; Figures 2(d)-(f)). Like the examples in the present specification, Imai *et al.* used an ACE2 knockout mouse model. Imai *et al.* also used a lung elastance assay as described in the specification (*see* p. 40, ln. 12-20), and

a route of administration (intraperitoneal injection) as disclosed in the specification (*see* p. 21, ln. 27-30). Thus, Imai *et al.* demonstrated a method of treating an ACE2 decreased state by administering to a mammal a therapeutically effective amount of ACE2 polypeptide. The results of Imai *et al.* also provide additional evidence of the conserved function of ACE2 and the renin-angiotensin system because a human ACE2 protein was able to complement ACE2 function in mice.

8. Studies by my research group at University Hospital Innsbruck provide additional evidence that the currently claimed method can be practiced without undue experimentation. Attached to this declaration as Exhibit 4 is a research report ("Research Report") of work conducted by Alexander Löckinger and Benedikt Treml of my research group. This work was pharmacologically evaluated by Manfred Schuster and Hans Loibner of the firm Apeiron for which this work was conducted. This report describes a study of recombinant human soluble ACE2 (rhACE2) in a piglet acute respiratory distress syndrome (ARDS) model.

9. The piglet ARDS model is a generally accepted animal model for the study of acute respiratory distress syndrome. ARDS was induced by continuous infusion of 50 μg/kg lipopolysaccharide (LPS) for the duration of the experiment and further 1 - 3 LPS bolus injections of 50 μg/kg each (Research Report, p. 1, para. 2). The average LPS quantity administered was 319 μg/kg and nearly equally distributed over both groups (Research Report, p. 1, para. 2). An ACE2 polypeptide, rhACE2, was administered as a central venous bolus injection at a dose of 100 μg/kg following the last LPS bolus injection and 120 minutes from the start of the continuous LPS infusion (Research Report, p. 1, para. 2). Intravenous injection is a route of administration disclosed in the present specification (*see* p. 21, ln. 27-30). The rhACE2 bolus

injections were well tolerated and did not show any apparent side effects (Research Report, p. 1, para. 3). Several hemodynamic parameters as well as pharmacokinetics were investigated in the piglet ARDS model.

10. Initial studies showed that rhACE2 had a half-life time in the piglet ARDS model of 77 minutes (Research Report, p. 1, para. 4; Figure 1).

11. Following treatment with rhACE2, pulmonary arterial pressure (PAP) stabilized or even decreased slightly in the rhACE2 treated group, while the control group showed a nearly 15% increase in PAP (Research Report, p. 2, para. 1; Figure 2). Systolic arterial pressure (SAP) was also measured. The control group showed an increase in SAP up to 12%, whereas after rhACE2 injection a stabilization and 5% decrease in SAP was observed (Research Report, p. 2, para. 2; Figure 3). The difference between the control and rhACE2 treatment groups was significant (Research Report, p. 2, para. 2).

12. Oxygen concentration was measured in arterial and venous blood samples taken from the piglets every 30 minutes (Research Report, p. 3, para. 1). Values are displayed in Figure 4 of the Research Report. Oxygen concentration decreased in arterial and venous blood in both groups (Research Report, p. 3, para. 1; Figure 4). A potential stabilization of arterial as well as venous oxygen concentration in the group receiving rhACE2, which might be observed first in the venous, later in the arterial blood, did not reach statistical significance in this study and will have to be confirmed in further experiments.

13. In view of the rat and mouse animal model studies in the specification showing the role of ACE2 in cardiac, lung, and kidney diseases; the disclosed similarities of the rat, mouse, and

human ACE2 structure and function; and the teaching in the specification that an ACE2 decreased state, such as cardiac, lung, and kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2; I believe that at the time the application was filed the inventors of the present application were in possession of a method of treating an ACE2 decreased state comprising administering to a mammal having hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis, renal failure, and/or lung disease, a therapeutically effective amount of an ACE2 polypeptide.

14. Furthermore, in view of the rat and mouse animal model studies in the specification showing the role of ACE2 in cardiac, lung, and kidney diseases; the disclosed similarities of the rat, mouse, and human ACE2 structure and function; and the teaching in the specification that an ACE2 decreased state, such as cardiac, lung, and kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2; I believe that the currently claimed method of treatment could be practiced without undue experimentation in any mammal in need of such treatment. This is confirmed by the demonstration by Imai *et al.* that injecting ACE2 knockout mice or acid-treated wild-type mice with a rhACE2 protein protected the mice from severe acute lung injury; and the study in the piglet ARDS model showing that rhACE2 protein therapy stabilized or even decreased both pulmonary arterial pressure and systolic arterial pressure.

15. I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 03-20-07

Nikolaus Neu, M.D.